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Human adipose derived mesenchymal stem cells (ADMSC) for cartilage repair: in vitro use of Gelforcel® hydrogel

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Purpose: Agarose alginate hydrogel (Gelforcel®) was used successfully as a matrix for autologous chondrocyte implantation. It presents interesting scaffold characteristics for tissue engineering: 3D environment allowing differentiation, stabilisation and homogenous distribution of the cells. The potential of the ADMSC included in this hydrogel instead of chondrocytes was evaluated in vitro in this work.
Methods and Materials: Cells are extracted from adipose tissue and then cultivated at different initial plating density in a plastic culture flask in expansion medium (containing of 10% calf bovin serum and 1ng/ml fibroblast growth factor 2). After 1 week, cultivated ADMSC were analyzed for phenotypic studies by flow cytometry with detection of specific ADMSC markers (CD73, CD90, CD105). Functionality was studied by: • Clonogenic tests: Quantification of CFU-f (colony forming unit cells), • Phenotype tests: culture of ADMSC included in gel in chondrogenic medium. After 3 weeks, gels were analyzed by immunohistochemistry (aggrecan and collagen II).
Results: Optimal plating density correspond to 10.103 ADMSC/cm2. ADMSC phenotype was positive for CD73, CD90, CD105 markers. Expanded ADMSC presented a high clonogenicity at least equivalent to bone marrow (CFU number). ADMSC included in hydrogel and incubated with chondrogenic medium showed type II collagen and aggrecan expression.
Conclusions: The use of ADMSC included in Gelforcel shows in vitro promising results which would make possible to avoid the articular biopsy

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Isolation, expansion and chondroblastic/osteoblastic differentiation of mesenchymal stem cells obtained by density gadient in surgical room.

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Purpose: Mesenchymal Stem Cells (MSCs) are capable of differentiating into chondroblast and osteoblast. It raised the possibility of use MSCs to repair osteochondral defects. Ideally for further applications in Cellular Therapy, the MSCs must be obtained during the surgical procedure. Purpose: To evaluate the ability to obtain human bone marrow derived MSCs (hBMMSCs) in a surgical room by Density Gradient (DG).
Methods and Materials: Isolation of hBMMSCs: In a surgical time an iliac puncture was performed and bone marrow aspirated was collected (15 ml). DG was performed and a mononuclear cell level (MCL) was obtained. To determine the presence of MSCs in the MCL, this portion was expanded and differentiated in our laboratory. Osteogenic Differentiation Medium: dexamethasone, b-glycerophosphate, and ascorbic acid-2-phosphate. Osteogenic differentiation was evaluated by alkaline phosphatase (AP) and histochemistry. Chondrogenic Differentiation Medium: ITS+Premix, TGF-b1 and ascorbic acid. Chondrogenioc differentiation was evaluated by Western blot for collagen
Results: DG: Successful, 3 ml of MCL was obtained. Isolation of hBMMSCs: mononuclear cells concentration: 9.000.000 cells/ml. Time to achieve confluence (80%): 18 days. MSCs at the end of culture: 28.000 cells/cm2. Osteoblastic Differentiation: Time to differentiation: 14 days. AP histochemestry: positive. Chondrogenic Differentiation: Western blot collagen II: positive.
Conclusions: Is possible to obtain MSCs by DG in surgical room. These MSCs had the osteogenic and chondrogenic capacity. This methodology could be applied to the cellular therapy in the clinical setting.

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A new methodology to isolate mesenchymal stem cells from human adipose tissue

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Purpose: Adipose Tissue is a promising source of Mesenchymal Stem Cells (MSCs). They can be obtained by a minimal invasive method and in large quantities. Purpose: To evaluate a new methodology to isolate human Adipose Tissue Mesenchymal Stem Cells (hATMSCs).
Methods and Materials: Samples of Human Adipose Tissue (hAT) (50 ml) were obtained from women undergoing elective liposuction. Samples were washed for 1 hour with DMEM. Then, tissues were digested at 37° C for 40 minutes with 0.04% collagenase type I under permanent shaking. After 5 minutes of centrifugation three phases were obtained: stromal phase (SP) / upper part of the tube, liquid phase (LP) / middle area and lax phase at the bottom. The LP was separated and expansive medium (DMEM, fetal bovine serum and antibiotic) was added to inactivate collagenase. Then, the LP was centrifuged at 1.300 rpm for 5 min and a cellular pellet was obtained. The cellular pellet was plated at concentration of 10^5 cells/cm^2 and incubated at 37°C/5% CO~2~ in expansive medium. To determinate the presence of MSCs, osteogenic and chondrogenic differentiation was performed.
Results: Samples of 10 patients were processed (52,6±12ml). Cells were plated at a mean density of 84.145±48.718cells/cm^2. Time to achieve confluence (80%): 16±5days. MSCs at the end of culture: 32.000±3.500cells/cm2. Osteogenic differentiation: Successful (alkaline phosphatase histochemestry: positive). Chondrogenic differentiation: Successful (western blot collagen II).
Conclusions: With this new methodology we were able to isolate and differentiate hATMSCs. This methodology could be useful for cellular therapy in the clinical setting.

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The effect of different sterilization processes on the mechanical properties and histomorphology of rat bone-patellar tendon-bones.

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Purpose: The purpose of this study was to evaluate the effect of different sterilization processes on the mechanical properties and histomorphology of rat bone-patellar tendon-bones (BTBs).
Methods and Materials: We harvested rat BTBs and divided them into three groups; (a) fresh group, (b) cryopreserved group, which were stored at -80 degrees C for 3 weeks, and (c) warmed group, which were heated at 80 degrees C for 10 minutes. Mechanical properties were evaluated by tensile testing. Histomorphological examination was performed by light microscopy and transmission electron microscopy.
Results: 1) Mechanical properties: The tensile strength and Young's modulus of the warmed group were significantly less than those of the fresh and cryopreserved groups ($p<0.05$). The strain at failure of the warmed group was significantly greater than that of the other two groups ($p<0.05$). The tensile strength, strain at failure and Young's modulus showed no significant differences between the fresh and cryopreserved groups. 2) Histomorphological evaluation: Light microscopy showed that the structure of collagen fibers in the warmed group was destroyed. When cryopreserved BTBs were thawed at room temperature, light and transmission electron microscopy showed no differences between the fresh group and the cryopreserved group. However, when cryopreserved BTBs were processed by the freeze-substitution method, many spaces were observed between the collagenous fibrils and these spaces seemed to squeeze and transform interfibrillar substances. These results suggest that ice crystal formation in the collagen fibers influences the structure of collagen fibers and interfibrillar substances.
Conclusions: The process of heating to a high temperature is not appropriate for sterilization of BTBs. In the cryopreserved BTB, ice crystal formation in the collagen fibers influences the structure of collagen fibers and interfibrillar substances.